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Cancer Stem Cells — Perspectives and How to Target Them

Zhaopeng Tang, Qianfeng Wang, Sarah Shigdar, Wei Duan and Dongxi Xiang

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Abstract

Cancer stem cell is a progressive concept moving forward to interpret the hard-to-cure nature of cancer and the relevant behavior in response to clinical therapies. Despite the remaining debates regarding the existence of cancer stem cells, the cancer stem cell model provides a potential approach for advanced innovative therapies targeting the "roots" of cancer, which has enhanced treatment outcomes. This chapter summarizes advanced perspectives in the field of cancer stem cell research, including experimental strategies for targeting these cells, highlights challenges of this theory, and explores feasible therapeutic strategies for overcoming the intrinsic resistance of cancer stem cells to clinical treatment.

Keywords: Cancer stem cell, stemness, chemoresistance, targeted therapy

1. Introduction

Cancer remains one of the leading causes of human death, and even though extraordinary efforts and budget have been spent, clinical trials in the eradication or control of cancer progression have generally created disappointing outcomes [1, 2]. Cancer develops originally from normal cells through the accumulation of multiple genetic alterations that ultimately convert to malignant phenotypes [3, 4]. Despite a better understanding of cancer biology and evolutionary genomic characteristics, translating these achievements into feasible and successful clinical outcomes continues to be a problem [5]. One attractive theory, the concept of cancer stem cells (CSCs), being explored recently in cancer research may hold the answer [6]. This chapter summarizes the major characteristics of CSCs and highlights several key approaches used for CSC study, where they could be of help for efficient CSC-targeted therapy.

2. CSC: An involving concept moving forward

2.1. Cancer genetic evolution

Cancer biology and genomics have increasingly validated cancer as a complex adaptive system. This landmark perspective was introduced by Peter Nowell in 1976, who viewed cancer development as an evolutionary process driven by stepwise mutations with sequential, subclonal selection at the nuclei acid level, a theory that is similar to Darwinian natural selection [7]. This theory includes several key aspects. (1) Genetic instability: cells possess a battery of mechanisms to preserve their DNA structural integrity. Cellular genetic structural varies (deletions, duplications, and rearrangements) or DNA point mutations can initiate cell biological changes that may lead to tumor formation. Thus, the perturbation of mechanisms controlling genomic stability is responsible for the oncogenic processes [8]. (2) Error-prone repair processes and a genotoxic exposure could result in particular mutational spectra of cancer cells, including cigarette carcinogens, ultraviolet light, and chemotherapeutics. (3) Recurrent, mutation specific traits in cancer can potentially affect clonal selection [9] (Figure 1).
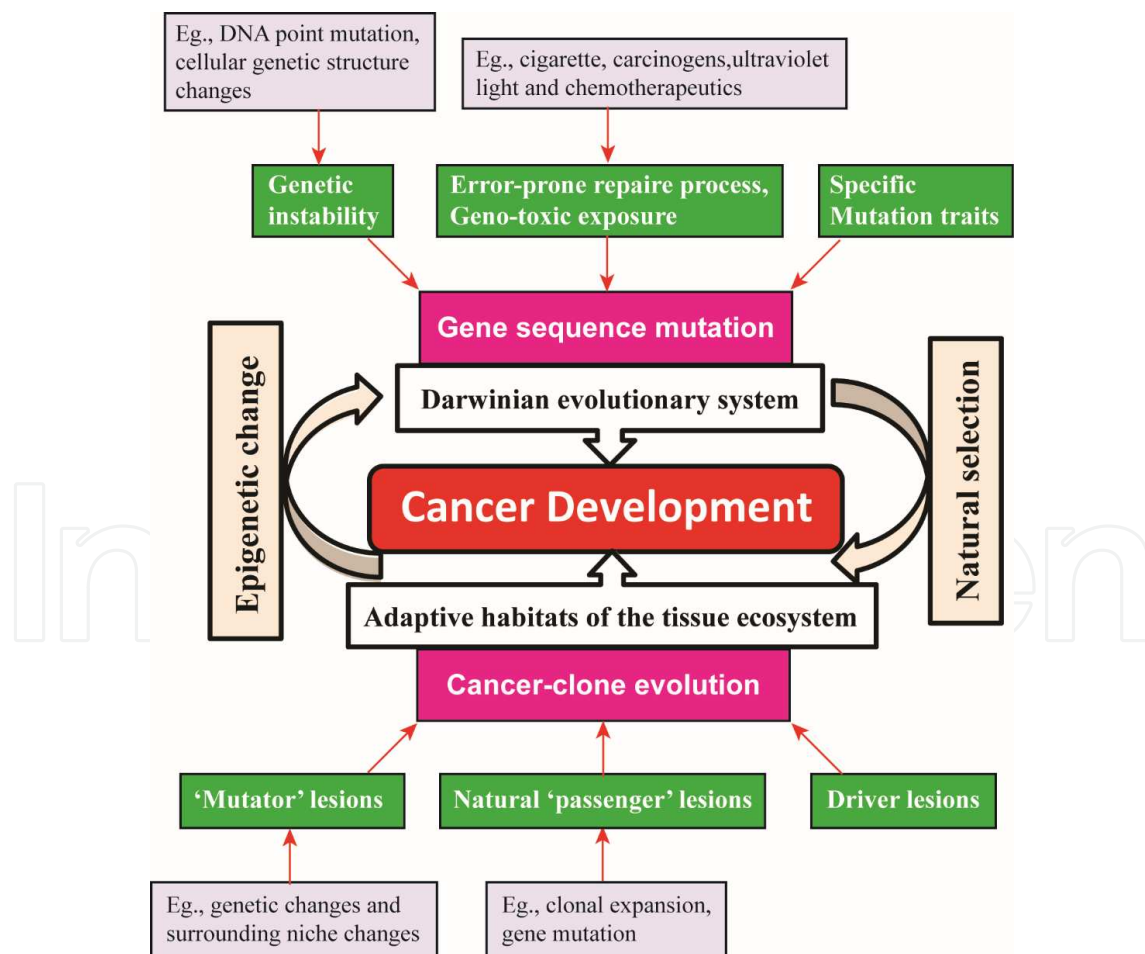

Figure 1. Genetic diversification and clonal dynamics of cancer development.

The microenvironment surrounding a tumor complexes with multiple cellular components that provide an adaptive landscape and necessary elements for natural selection of cancer growth [10]. The interaction between cancer cells and their surroundings is mutually beneficial [11]. Cancer cells can reset the extracellular environment to be specialized niches for further cancer growth and migration. The cancer niche in turn provides external signals for cancer-clone expansion and tumor cell survival and proliferation. The spatial heterogeneity is critical for evolving cancer cell to be a malignant phenotype and beneficial for cell migration and distant invasion [12]. During the process of cancer-clone expansion, migrating cells invade new habitats where they face new selective pressures, thereby increasing the rate of further cancer mutation [13].

The cancer niche is an unclosed system. In addition to the tissue positions and associated etiological environmental exposure of patients, the tissue microecosystem is manipulated by multiple systemic or external factors [14]. Genotoxic exposures, such as ultraviolet light, pathogenic infection, and long-term dietary habits, are able to modulate energy metabolism. They are speculated to be the primary etiological causes of tumor initiation and further evolution of cancer clones [14]. Cancer cell ecosystems can be altered following clinical treatment, in which most cancer cells together with healthy tissues are killed under intensive therapies. However, some specialized niches protect cells from cancer treatment, where variant or therapy-resistant cancer cells emerge [15]. On the way of cancer progression and tumor recurrence after therapy, the primary unit of selection is a specialized cell, known as CSC, which possesses extensive self-renewal potential (Figure 2).
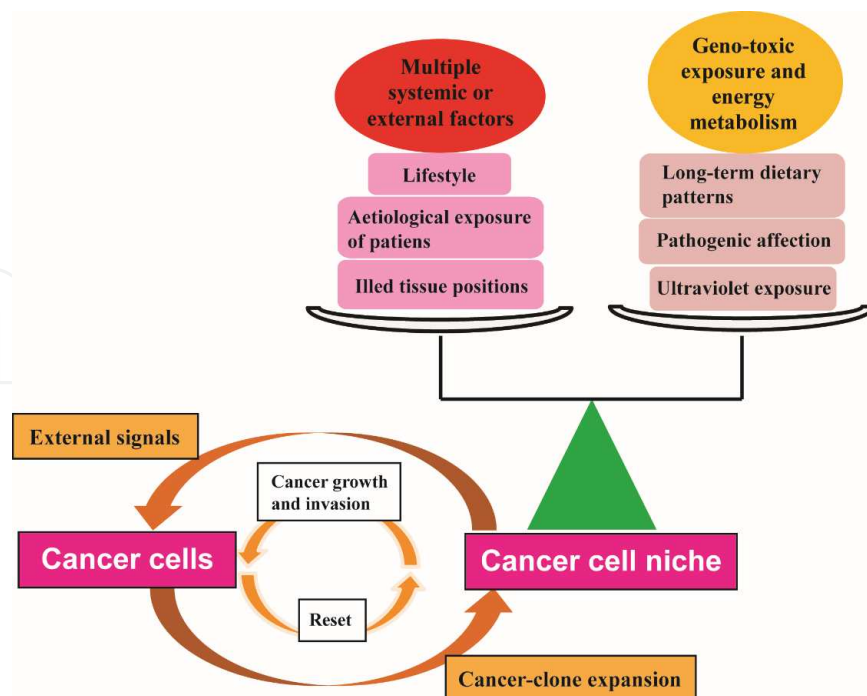

Figure 2. The cancer ecosystem.

2.2. Cancer stem cell model

Cancer research was previously dominated by the clonal evolution model, also known as a conventional stochastic model, a concept whereby all cells within a tumor have equal potential to propagate and maintain a tumor following stepwise genetic and/or epigenetic changes, but they are hard to identify the tumorigenic subset [7, 16–19]. Recently, accumulated studies suggest cancers as hierarchical organizations, which is the basis of the hierarchical or CSC model [20]. In the CSC model, only a small subset of cancer cells possesses the ability to self-renew, differentiates, and reforms a tumor. CSCs, as "roots of cancer" operating in a hierarchical fashion, are defined by their abilities [20–24] (1) to form new tumors with high efficiency that histologically resembles the original tumors when xenotransplanted into immunodeficient mice, (2) to generate descendant cells possessing unlimited self-renewal (regeneration) potential but uncommitted differentiation options, and (3) to generate large populations of differentiated offspring and progenitor cells that exit the stem cell state and lose the ability to self-renew, thus no longer possessing tumorigenic potential. Such differentiated daughter cells can undergo limited rounds of cell division because they lack the intrinsic clonogenic characteristics that are essential for tumor initiation and long-term progression of the malignancy. The increasingly widespread attraction of the CSC model is due to the fact that it can provide a plausible account for poorly understood clinical phenomena, such as therapy resistance, as CSCs can resume growth and contribute to a new relapse after therapy suspension. It is critical to appreciate that both the stochastic and CSC models share the same conviction that only a small population of cancer cells are capable of maintaining cancer. The main difference is that CSCs within the CSC model are characterized with the help of distinct cellular phenotypes [20].

Critically, the CSC model stands on the basis that CSCs are reliable and stable over time, and their unique traits could not be obtained by differentiated descendants. It is worth to note that the persuading of CSC model has been accompanying with intense debates amongst cancer researchers. As evident from a new concept, the dynamic CSC model suggests that the CSC phenotype is much more fluid than previously predicted and can be regulated by external signals [25, 26]. Thus, not only can CSCs self-renew to create new progeny and differentiate to non-CSCs but the de-differentiation of non-CSCs to CSCs can also occur and thus return to the malignancy growth cycle [26–28]. This latest finding has significant implications for oncology in that future effective anticancer therapeutic strategies should aim at targeting both CSCs and non-CSCs (Figure 3).
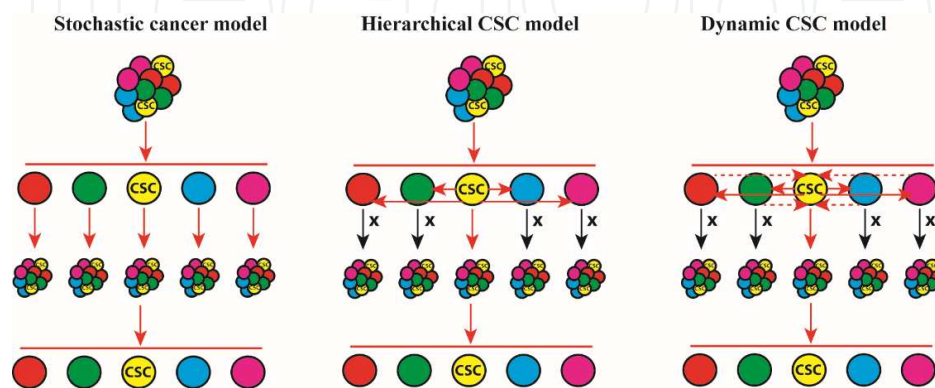

Figure 3. Stochastic cancer model versus hierarchical and dynamic CSC models of tumor heterogeneity.

3. Challenges and opportunities the CSC theory faces

Several challenges the CSC theory is now facing should be addressed before this concept can fetch benefits for the clinically relevant entity [29].

3.1. Frequency of CSCs

One challenge that is intensely debated is how many CSCs exist within a tumor. Some studies suggested that the frequency of CSCs is less than 1 in every 1000 cells in a tumor, which supports the hierarchical status between CSCs and non-CSCs [30, 31]. However, recent evidences reported that the number of cells that possess intrinsic tumorigenic potential is relatively higher [32, 33]. Hepatocytes possess the potential of self-renewal and unlimited proliferation under certain conditions [34, 35]. It is shown that various colorectal cancers do not follow the same hierarchy as their CSC frequencies vary [36]. More importantly, the prediction of the number of CSCs in a particular tumor type depends on multiple manual factors, including the experimental procedure designed, the choice of cell surface markers, and the types of immunodeficient mice into which the CSCs were injected. Chiba et al. used the technique of side population (SP) analysis to detect the subpopulations that act CSC functions and revealed that SP cells possess abilities of high proliferation and antiapoptosis in both Huh7 and PLC/PRF/5 cells with a percentage of 0.25% and 0.8%, respectively [37]. Kimura et al. determined the frequency of CSCs by analyzing cell surface markers and the percentage of CD133+ cells in Huh7 and PLC/PRF/5 were 2.7% and 23.5%, which is totally different with the number obtained on the base of EpCAM-positive cells, with a proportion of 89.1% and 40.7%, respectively [38]. However, Cao et al. found the CSC frequency of PLC/PRF/5 cells was 8.8% by using the sphere-forming assay [39]. Thus, considering a tumor as a hierarchical malignancy has intrinsic limitations, as a large population of cancer cells cooperate and contribute to tumor growth, which is consistent with the conventional nonhierarchical model of malignancies [40].

3.2. Therapy resistance of CSCs

The failure of cancer initial treatment is normally explained due to the presence of a subset of resistant cancer cells. CSCs are supposed to be one of such cells; they are logically resistant to traditional chemotherapies owing to their "stem-like" features, including enhanced abilities of DNA damage repair and cellular detoxification capacity via elevated aldehyde dehydrogenase (ALDH) activity, increased expression of enhancement of ATP-binding cassette (ABC) surface transporters, and their nature quiescence [41–43]. CSCs with an up-regulation of antiapoptotic molecules or a high expression of drug efflux pumps respond quite differently when exposed to drugs; they can both survive after cancer treatment [44]. The rapid relapse of the malignancy suggests that the clonogenic core of the cancer was not effectively targeted, and this might be due to the de-differentiation of non-CSCs to CSCs [45]. Even though CSCs are believed to be the most promising candidates for overcoming chemoresistance and tumor recurrence, both CSCs and non-CSCs should be targeted eventually.

3.3. Isolation of CSCs

The isolation and subsequent assessment of CSCs are clearly a rapidly developing area in which diverse strategies were incorporated for obtaining higher CSC purities. One continual concern after isolation assays is that whether the proposed CSCs separated from the bulk of the tumor can still possess the intrinsic cellular properties. The selected CSCs require the distinct phenotypes that can generate a similar malignancy to the parental tumor when transplanted into immunodeficient mice [46]. The subsequent challenge is to optimize experimental procedures to allow xenotransplantation of viable single suspension cells into animals.

3.4. Targeting "confirmed" CSCs

Due to the relevance of cancer development, CSCs are ideal targets for molecular-directed therapies. The limitation is that CSCs are typically present at very low levels. In addition, CSC markers overlap with normal stem cells or between CSCs [47]. These drawbacks indicate that the identification of CSC markers for guiding molecular therapeutics is still in its infancy. Many CSC markers are not strictly CSC antigens since they present on normal cancer cells as well, but they are molecules that support CSCs in their niche.

The characterization of CSCs has led to some experimentally "confirmed" markers or markers involved in the process of targeting or interaction with CSCs. Epithelial cell adhesion molecule (EpCAM) is one of the most highly expressed tumor-associated markers, being found in a broad range of epithelial cancers. EpCAM has been "rediscovered" as a CSC antigen in breast, colon, prostate, and pancreatic cancers [48–50]. In studies on colon cancer tumorigenicity, EpCAMhighCD44+ was considered as a robust marker of more "stem-like" subpopulations. Also, recruiting effector cells to tumors using an anti-EpCAM/anti-CD3 bispecific antibody has been shown to be a promising strategy in the treatment of cancer [51].

In addition to cell-surface markers that are frequently utilized to identify CSCs, the high activity of signal transduction routes can also contribute to CSCs features [52]. The self-renewal capacity of CSCs leads to a long-term clonogenicity mutation, which might be effectively therapeutically targeted. The activation of intracellular signaling pathways associated with the self-renew of CSCs, including Wnt pathway, Hedgehog (Hh) pathway, and Notch pathway, can stimulate a more immature tumor phenotype, facilitate tumor invasion, and promote therapy resistance [53]. The interference of such pathways that promote CSC function might provide an effective therapeutic window for drugs in the war against cancer. These stem cell-associated surface marker proteins and pathways are promising targets for anticancer drug development

4. Approaches used for studying CSCs

The primary challenge to study CSCs is the ability to identify and investigate CSCs in laboratories using both in vitro and in vivo assays. This section addresses the methodologies that have been widely used for isolation and characterization of CSCs.

4.1. In vivo xenotransplantation and limiting dilution assay: The gold standard

Limiting dilution assays (LDAs) have been used in a broad variety of biological fields. LDA is an experimental technique that attempts to quantify the frequency of biological particles that perform a particular function within a larger mixed population [54]. The aim of LDA is to obtain highly precise data at the macro level. To achieve this goal, several conditions must be considered [55, 56]. First, the cells should be Poisson distributed. Second, the assay should be designed with maximum information containing both negative and positive cultures. Moreover, the conditions must be performed at the state that the response of a single limiting particle can be detected. Thus, the culture conditions should be uniform in all paralleled groups and wells [56].

The ability to determine the number of CSCs is a critical step for the success of in vivo transplantation. However, there has been little or no attempt to standardize this approach [57]. In all conditions tested, the statistical behavior of the system (Es) and changes in condition over time and statistical noise were the two major contributors to overall uncertainty [57]. However, as expected from statistical principles, Es declines with increasing number of replicates analyzed. Also, increasing the number of replicates beyond 96 is unlikely to provide substantial decreases in error, and fewer wells can be used to obtain results with similar precision. Another requirement that arises in stem cell research is the need to accommodate small numbers of replicates in a statistically consistent and defensible manner. Operator error, mouse-to-mouse error, and other errors appear to play minor roles. These findings establish parameters that contribute to the variability of LDA and provide strategies for the optimization and interpretation of the LDA-based CSC estimates. To this end, extreme limiting dilution analysis (ELDA) has emerged as the preferred method that is based on sound statistical principle and methodologies [58]. ELDA works well when the number of replicates is small.

The CSC concept brings essential predictions of cancer research including susceptibility to chemotherapy, aggressiveness of the disease, and the pace of recurrence that may largely be influenced by the functional properties of CSCs [48, 49, 59–61]. To functionally measure CSC potential, the operational assay of evaluating CSCs has been developed. The creation of an array of genetically modified immunodeficient mice enabling analogous xenograft experiments can be used to determine and quantify cells that obtain tumor-initiating activity in human tumors [48, 49, 59–61]. These xenograft models are considered the "gold standard" in CSC research; they are able to guide the biology and therapeutic responses of human CSCs.

The enumeration of CSCs has been performed using phenotypic markers in in vitro limiting dilution transplantation assays. Some key aspects should be considered [32]: (1) inefficient engraftment in mice may result in underestimation of the actual CSC frequency; (2) phenotypic characterization of CSCs may lead to different rates depending on the antibody combinations used, and the use of specific antibodies may induce immune-mediated clearance of CSCs [48]; (3) the approach of tumor implantation (e.g., intravenously, intrafemorally) critically defines engraftment and affects the export of stem cell frequency [62]; and (4) the kind of mouse strain used as tumor recipient and the degree of mouse immunodeficiency are other factors need to be considered [32]. It is reported that the use of adjuvants/culture supplementary (e.g., Matrigel or growth factors) influences not only the measured frequency of CSCs but their phenotypes as well [30, 32].

4.2. The side population assay

In addition to the isolation and identification of CSCs through a set of cell surface markers (such as CD44+/CD24low/lin-/ALDH+, CD44+/CD24low, EpCAM+/CD44+/CD24–, or CD34+/CD38–). The SP discrimination assay is another flow cytometry method used to detect stem cells based on the properties of the Hoechst dye efflux via the ABC transporters [63]. The SP assay has been used to identify stem cell and progenitor populations in various tissues [64, 65]. However, several reports have stated that dye efflux is not a universal property of all stem cells although SPs are much abundant within stem cells [66]. SPs are not just restricted to the stem cell phenotypes as ABC transporters are also expressed by specialized cells in certain organs [67]. The ABC transporters are believed to play a key role in those tissues, protecting the cytotoxic effects of toxins [68]. However, the identification of CSCs has raised interests in the SP assay, and SP subpopulations isolated from some cancers possess capabilities of drug-resistant, self-renewal, and tumorigenicity when transplanted into immunocompromised mice [63]. The SP assay can play an extremely valuable role in the primary isolation and identification of potential stem/progenitor cells, when specific cell surface markers were absent.

4.3. Clonal sphere formation assay and proliferation (invasion assay) assay

The clonal sphere formation assay is another functional method used for the identification of CSCs and their purification from the rest of neighboring cells [69, 70]. This assay begins with reliable single cell suspensions originating from primary tumors or established cancer cell lines. Serial dilutions of the single cancer cell suspensions are seeded on ultra-low attachment substrata in the presence of serum-free media with growth factors [71]. CSCs can grow into 3D nonadherent structures called spheres, while non-CSCs cannot survive in such culture conditions. The results of the assay may become more reliable if the spheres are serially passaged. The self-renewal capacity of sphere-generating cells can be estimated by evaluating the sphere formation frequency through LDA and measuring sphere size [72]. The sphere formation assay has been used to identify adult stem cells or CSCs from a number of tissues, including mammary gland, brain, skin, and human melanoma [73–76].

4.4. Enzymatic assay of cell-surface markers

CSCs can be isolated by the enzymatic activities (ALDH activity) [77, 78]. The measurement of ALDH activity by ALDEFLUOR staining is useful to screen tumor cells for resistance to alkylating agents and to identify heterogeneity within tumor cells [79]. The ALDH+ population is consistent with CSC characteristics, generating tumors that recapitulate the phenotypic heterogeneity of the initial tumor. The measurement of ALDH populations revealed that the ALDH+ cells were capable of self-renewing into both ALDH+ and ALDH− cells [80]. A recent study in melanoma described both ALDH+ and ALDH− cells derived from patient biopsies (100 or 2000 cells, respectively) were able to efficiently form tumors. It is also reported that ALDH cells alone may not be sufficient for CSC selection [32]. Despite this, studies in breast cancer combining other CSC surface markers with Aldefluor have improved tumorigenic enrichment and this combination may prove to be a better strategy for enrichment of CSCs [32].

4.5. Slow-cycling population assay

The slow-cycling population assay can be used to distinguish CSCs from progenitor cells, which determines the incorporation of labeled precursor of nucleotides for cellular DNA synthesis [81]. The slow-cycling cells, such as CSCs, maintaining sufficient labels allow their detection by the anti-BrdU antibody staining or radioactive label [82]. Some technical issues impede the accuracy of BrdU detection: (1) it is hard to demonstrate that cells have a similar cycling in vitro and in vivo because of the destructive nature of the BrdU detection procedure, (2) it is not expected that all the stem cells can be equally labeled because the BrdU incorporation occurs only during the S phase of cell cycle, and (3) the label retention depends on the length of the cell cycle, while the later status appears to alter as the organ matures [83]. Thus, quantification of stem cells through this assay requires further confirmation through other methods.

4.6. Lineage labeling assay

The lineage labeling assay was initially developed for understanding and tracing the biological developments of a cell, including the identification of cell origin, measuring lineage relationships, and determination of division patterns [84]. In this assay, cells are labeled (usually with a fluorescent dye or tag), followed by tracing in vitro and in vivo. There are several inherent limitations with this technique: (1) the experimental procedure can damage cells or some cells may have weak and/or transient expression of markers and unrestricted clonality [84] and (2) with whole-mount labeling, it is not possible to identify spatial relationships between stem cells and its transit amplifying progeny [85]. In this case, targeted cells are noninvasively labeled in their native environment, and the development of a progenitor cell and a composition of their lineages can be followed. It remains unclear whether the combination of these techniques can be extended to identify CSCs and metastatic initiating stem/progenitor cells.

While there has been a hot debate in recent years as to whether the CSC theory is correct, very recent lineage tracing studies have provided proof that a relatively small number of cells are capable of generating and maintaining a tumor. These studies used fluorescently labeled tumor cells and showed that the quiescent cells remained after further in vivo passages [86]. Furthermore, following treatment, these quiescent cells started to proliferate and generate proliferative progenitor cells that were capable of maintaining a tumor [87–89]. By eliminating these quiescent cells, tumor growth was impaired. These results link back to the theory that leaving a small population of CSCs after the conventional treatment lead to a recurrence of the tumor. Therefore, it is necessary to eliminate both populations of cells within a tumor to produce effective anticancer therapeutic strategies [90].

4.7. CSC epigenome and next-generation sequencing

Whole genome sequencing has made tremendous contributions to cancer research, which enables researchers to discover and understand the rules of cancer development at the nucleic acid level [91, 92]. However, the genome instability and genetic variants limit to demonstrate cancer progression. It was speculated that there exist a senior layer of information, besides

genome sequence for regulating differential gene expressions and thus determining cancer evolving. This concept was early prospected by Conrad Hal Waddington in 1942 and subsequently described as "epigenetic control system" by Nanney in 1958 [93, 94]. Epigenetics primarily refers to the study of chromosome variations that modulate gene transcription without alterations in the DNA sequences [95, 96]. Epigenome contains genetic information, not as stable as genome, representing the cellular epigenetic state varying with influence from external factors. Mechanisms produce such changes mainly include DNA methylation, histone modifications, nucleosome positioning, and chromatin remodeling [96]. These epigenetic regulation mechanisms are reported to be closely related with the gaining of stem cell-specific properties, whereby contributing to tumor inheterogeneous [97, 98]. Epigenetic alterations would offer survival benefits in CSC subpopulation which promotes the genetic expression to the self-renewal state, resulting in tumor initiation and further progression [99, 100]. The relevance of the DNA methylation and histone modifications in CSC regulation, subsequently with advancing tumor growth, were recently illustrated in various cancer models [97, 99, 101].

Next-generation sequencing (NGS), also known as high-throughput sequencing, has revolutionized the study of genomics and opened a new chapter of the epigenome research of cancer and stem cell [102, 103]. Four mainly NGS-based approaches have been developed to identify epigenome research, including methylated DNA immunoprecipitation sequencing (MeDIP-Seq), whole genome bisulfite sequencing (WGBS), reduced representation bisulfite sequencing (RRBS), and chromatin immunoprecipitation sequencing (ChIP-Seq). Compared with the previous approach, the innovative advantages of NGS have significantly accelerated the sequence of DNA and RNA and promoted the scientific sightings in CSC epigenome research.

5. Therapeutic strategies used for targeting CSCs

Current failure of cancer treatment is normally due to the existence and functionalities of CSCs, which possess more chemoresistance than their non-CSC counterparts, to conventional treatments. Recently, multiple strategies have been developed for targeting and eradicating CSCs while sparing healthy tissues, thereby minimizing the patients to face therapy risks.

5.1. Targeting CSCs via surface markers

Since CSCs are attractive targets for anticancer treatment, the CSC-correlated cell surface proteins have been monitored extensively for identification, probable isolation and monitoring the variation of leukemic and solid CSCs in preclinical and clinical settings [104]. Studies using certain ligands or antibodies against CSC surface maker proteins, including EpCAM, CD47, CD44, CD90, CD133, IL-3R, immunoglobulin mucin TIM-3, ALDH+, and others, have advanced the therapeutic efficacy [105–108]. Limitations, such as the expression of such cell surface makers, may vary in different stages of cancer development; only a small subpopulation of CSCs standing in the summit among bunk of cancer cells, as well as overlapping of CSC-associated marker proteins with normal stem cells, impede the effectively targeting of CSCs [47]. For bench assays, it is recommended to employ functional studies such as in vivo

LDA upon xenotransplantation to ascertain CSCs, rather than solely be dependent on the incidence of a single or a combination of multiple CSC markers. However, in case of in vivo CSC-based therapy, the CSC surface marker proteins present one of the very few feasible options for targeting CSCs in animals and patients, given the practical and ethical restrictions related with lineage tracing and xenotransplantation.

Accumulated attractions have been grasped in the development of aptamers (also known as chemical antibodies) and monoclonal antibodies for targeting CSCs via targeting surface marker proteins. Shigdar et al. have isolated two RNA aptamers using SELEX for targeting CSC surface markers, EpCAM, and CD133 [109, 110]. These CSC-targeting aptamers internalize into target cells through receptor-mediated endocytosis, which is capable of circumventing ATP-binding cassette transporters that function multiple drug resistance in CSCs [26]. In addition, this newly developed EpCAM RNA aptamer is more sensitive than counterpart antibodies for detecting the surface EpCAM proteins in formalin-fixed paraffin-embedded primary breast cancers, with no nonspecific staining or cross-reactivity with non-EpCAM-expression tissues [111]. This shows the potential of aptamers for specific targeting of cancers but with minimized side effects observation. To optimize promising approaches by targeting cell surface markers might be one of the answers for tracing CSCs and eventually eradiating CSCs, thereby preventing patients from suffering from cancer attacking.

5.2. Targeting ATP-driven efflux transporters

The failure of cancer chemotherapy mainly results from increased efflux of anticancer drugs from cancer cells, resulting in the impairment of drug cellular entrance and consequent reduction of chemotherapeutic sensitivity [112]. A considerable root of multidrug resistance (MDR) involves the augmented expression of the ABC transporter superfamily, many of these transmembrane proteins are responsible for effluxion of various xenobiotics (including anticancer agents) from cancer intracellular membranes [112, 113]. The well-characterized MDR transporters consist with ABCB1 (MDR1 or P-glycoprotein), ABCC1 (MRP1), and ABCG2 (BCRP or MXR) [112, 114–116]. Although projects focusing on understanding the mechanisms of these ABC transporters behind chemotherapy failure have been broadly developed, clinical outcomes in these fields have been generally unsatisfactory [113, 117]. The recurrence of primary and metastatic cites can occur following the escaped chemotherapy, in which CSCs, possessing enhanced efflux of therapeutic agents through ABC transporters, have been proven to provide major contributions [114, 118, 119].

Due to the correlation of ABC transporters with CSC phenotypes, one approach circumventing CSC-based chemoresistance involves the use of specific molecular inhibitors against certain functions of individual MDR transporters [114]. Verapamil, an agent targeting general ABC transporters, has been moved to the clinical trials, but the further study was suspended due to the dose-limiting toxicity [120]. Thus, the more specific molecules targeting individual MDR transporters are required [121]. A considerable body of evidence has shown positive outcomes of overcoming CSC drug resistance by developing physical conjugation or chemical nanoparticle-functionalized drug delivery of anticancer agents. Chou et al. indicated that the conjugation of doxorubicin (DOX) into nanodiamonds reduced efflux of DOX in MDR1 overexpressing

cancer cells and facilitated the anticancer efficacy in DOX-resistant cancer models [122]. While CSC-related chemoresistance and tumor relapse arise mainly due to the increased expression of ABC transporters, the therapeutic strategies developed for specifically disturbing actions of ABC transporter proteins would be an attractive potential for targeting and eradicating CSCs.

6. Conclusions

The CSC concept is still an evolving model moving forward. Recent lineage tracing strategy has proven the existence and functions of CSCs in progression of solid tumors. CSCs with intrinsic activities of chemoresistance are the culprit in tumor recurrence and the root of treatment failures. Challenges remain on how to efficiently identify and target CSCs *in vivo* and monitor CSCs posttreatment. Cancer biology has proofed that traditional clonal evolution, and CSC models are not exclusive but co-exist with each other in tumorigenesis. Therefore, future elucidation of molecular mechanisms underlying CSC biology should open a new window of efficacious novel therapy strategies that eliminate both CSCs and non-CSCs.

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